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Immunoreactive Precipitation of C1 Inhibitor Protein from Plasma of Normal Subjects and of Patients with Hereditary Angioedema after Isoelectric Focusing

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Summary: C1-inhibitor is an acid glycoprotein, isoelectric point 3.5–3.6. Plasma of some patients with a variant form of hereditary angioedema contains high levels of functionless C1-inhibitor-albumin complex with an isoelectric point at 4.5–4.6. Therapy with Danazol, which increases C1-inhibitor levels, does not modify the isoelectric focusing pattern of such protein in patients with hereditary angioedema.

Immunreaktive Fällung von C1-Inhibitor-Protein aus Plasma Gesunder und von Patienten mit hereditärem Angioödem nach isoelektrischer Fokussierung

Zusammenfassung: C1-Inhibitor ist ein saures Glykoprotein mit einem isoelektrischen Punkt von 3,5–3,6. Plasma bestimmter Patienten mit einer Variante des hereditären Angioödems enthält hohe Konzentrationen eines funktionslosen C1-Inhibitor-Albumin-Komplexes mit einem isoelektrischen Punkt von 4,5–4,6. Therapie mit Danazol, das die C1-Inhibitor-Konzentration erhöht, führt zu keiner Änderung des isoelektrischen Fokussierungsmusters dieses Proteins bei Patienten mit hereditärem Angioödem.

Introduction

The inhibitor of activated first component (C1-inhibitor) is the major regulatory factor for the classical pathway of activation of human complement (1, 2). It has also been shown to inhibit several enzymes of haemostasis: activated *Hageman* factor (Factor XII), *Hageman* factor fragment, kallikrein, activated thromboplastin antecedent (Factor XI) and plasmin (3–6). Plasma from patients with hereditary angioedema was found to be deficient in C1-inhibitor function (inherited as an autosomal dominant trait), leading to the activation of complement. There are two forms of this disease. In the majority of the patients (type 1) there are very low levels of antigen concentration (0.026–0.052 g/l). However 15–20% of the patients have the variant form with normal (type IIa) or elevated (type IIb) antigen concentration of a dysfunctional protein (7–11). It has been demonstrated that in some hereditary angioedema patients with the variant form, both dysfunctional (94%) and functional proteins (6%) are present, and therapy with androgen derivatives increases both the proteins (12).

We have used isoelectric focusing to study C1-inhibitor in plasma from normal subjects and in patients with hereditary angioedema before and during androgen therapy, to determine the isoelectric point of the protein and evaluate the effects of such anabolic therapy on the characteristics of C1-inhibitor.

Materials and Methods

Normal and pathological specimens

Blood was drawn into plastic tubes containing 0.01 mol/l EDTA. It was centrifuged within 1 hour, at + 4 °C, and sam-

ples were stored in several aliquots at −80 °C until analysed. EDTA-plasma samples were taken from 10 healthy adult volunteers and from 18 hereditary angioedema patients:

10 with type I (C1-inhibitor antigen level: 0.026−0.052 g/l); 5 with type IIa (C1-inhibitor antigen level: 0.23−0.29 g/l) and 3 with type IIb (C1-inhibitor antigen level: 0.52−1 g/l).

C1-inhibitor functional activity was 10−20 U in all 18 patients.

Samples from hereditary angioedema patients were obtained before and during therapy with 400 mg/day Danazol for one month. At the sampling time, all the patients were asymptomatic.

Normal EDTA-plasmas were analysed after 2 hours and after storage for 2−4 month at −80 °C.

Chemicals and antiserum

Acrylamide, N'N'-methylenebisacrylamide and ampholines were obtained from SERVA-Feinbiochemica, Heidelberg (West Germany). The agarose (low-m = 0.13 ± 0.02) was a product of BIO-RAD Laboratories, Richmond Calif. (USA). Specific antisera against C1-inhibitor and albumin were obtained from Behringwerke, Marburg (West Germany).

Isoelectric focusing (IEF)-lying on the immunoelectrophoresis matrix

Thin layer IEF was performed as described by *Righetti* (13) with the following modifications: 4.4% polyacrylamide gel was obtained from a stock solution of 30% T 4% C, and contained 1.3 mol/l glycerol, 3.5% Ampholine 3−10 and 2.5% Ampholine 2−4; gels were polymerized on glass plates (U frame 1 mm) with 2.6 mmol/l tetramethylethylendiamine and 1.7 mmol/l ammonium persulphate. The anolyte was 1.0 mol/l phosphoric acid and the catholyte 1.0 mol/l sodium hydroxide.

The samples (18 µl) were applied to the cathode end side, in a well on the gel surface.

IEF were run on the LKB Multiphore apparatus, with a cooling system (temperature on the gel surface + 6−8 °C), for 4 hours, at 10 W constant power with voltage increasing from 300 to 1600 V.

After completion of the isoelectric focusing run, the pH gradient was determined and strips were cut in the migration area. They were then placed on 0.9% agarose−4% polyethyleneglycol gel plates containing monospecific antibody, and electrophoresis (3 mA for 12 hours) was performed. Tris/barbital buffer, ionic strengh 0.1, pH 8.6, was used for buffer vessels and gels. Agarose gel plates were stained with Coomassie Brillant Blue.

C1-inhibitor plasma levels estimation

The antigen level of C1-inhibitor was determined by single radial immunodiffusion on cellulose acetate strips (14); C1-inhibitor functional activity was determined by its inhibition of C1-esterase activity towards N-acetyl-*L*-tyrosine-ethyl ester (15).

C1-inhibitor reference values

C1-inhibitor antigen level (mean ± SD) = 0.26 ± 0.8 g/l
C1-inhibitor functional activity (mean ± SD) = 0.90 ± 0.12 U of C1-inhibitor
(U of C1-inhibitor = activity of 1 ml of fresh normal plasma)

Results

Plasma-EDTA samples from ten normal volunteers were studied by isoelectric focusing-immunoelectrophoresis after 24 hours and after 3−8 months storage at −80 °C. In all samples, C1-inhibitor protein focused at pH 3.5−3.6, giving only one peak in immunoelectrophoresis (fig. 1).

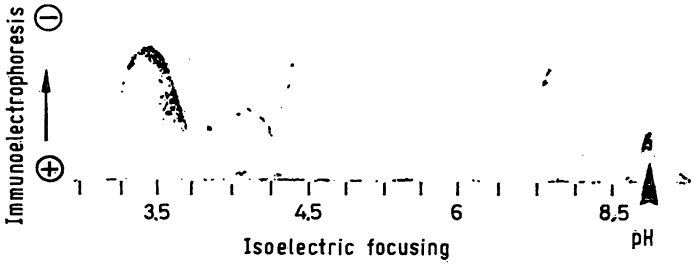

Fig. 1. Immunoelectrophoretic pattern after isoelectric focusing of C1-inhibitor protein in EDTA-plasma from a healthy subject.
Arrow indicates site of the sample application on the gel, before isoelectric focusing.

Plasma-EDTA samples from ten untreated patients with type I hereditary angioedema (C1-inhibitor antigen level less than 0.052 g/l) gave only one peak at pH 3.5−3.6, as observed for normal subjects. The same patients were treated with Danazol for one month (400 mg/day). The antigen concentration of C1-inhibitor increased to 0.078−0.10 g/l and functional activity to 35−45 U. There were no differences between C1-inhibitor in the plasma-EDTA before and during anabolic therapy (fig. 2).

Of the 8 patients with Type II hereditary angioedema, five (Type IIa) had C1-inhibitor antigen levels in the range 0.21−0.28 g/l and three (Type IIb) in the range 0.52−1 g/l. After one month of therapy with Danazol (400 mg/day), antigen levels had increased about 2 times while functional activity still ranged from 35 to 40 U.

Plasma-EDTA of Type IIa patients gave only one peak at pH 3.5−3.6, both before and during Danazol therapy (fig. 2).

In 3 untreated patients with Type IIb disease, plasma-EDTA exhibited a minor peak at pH 3.5−3.6, but 80−90% of the reacting material focused at pH 4.5−4.6 (fig. 3). This major peak could not be reproduced when crossed immunoelectrophoresis was performed with an intermediate gel containing anti-albumin (fig. 4). Although the precision of the quantitative estimation is rather low with this method, Danazol therapy apparently increased equally the amounts of protein focusing at both isoelectric points.

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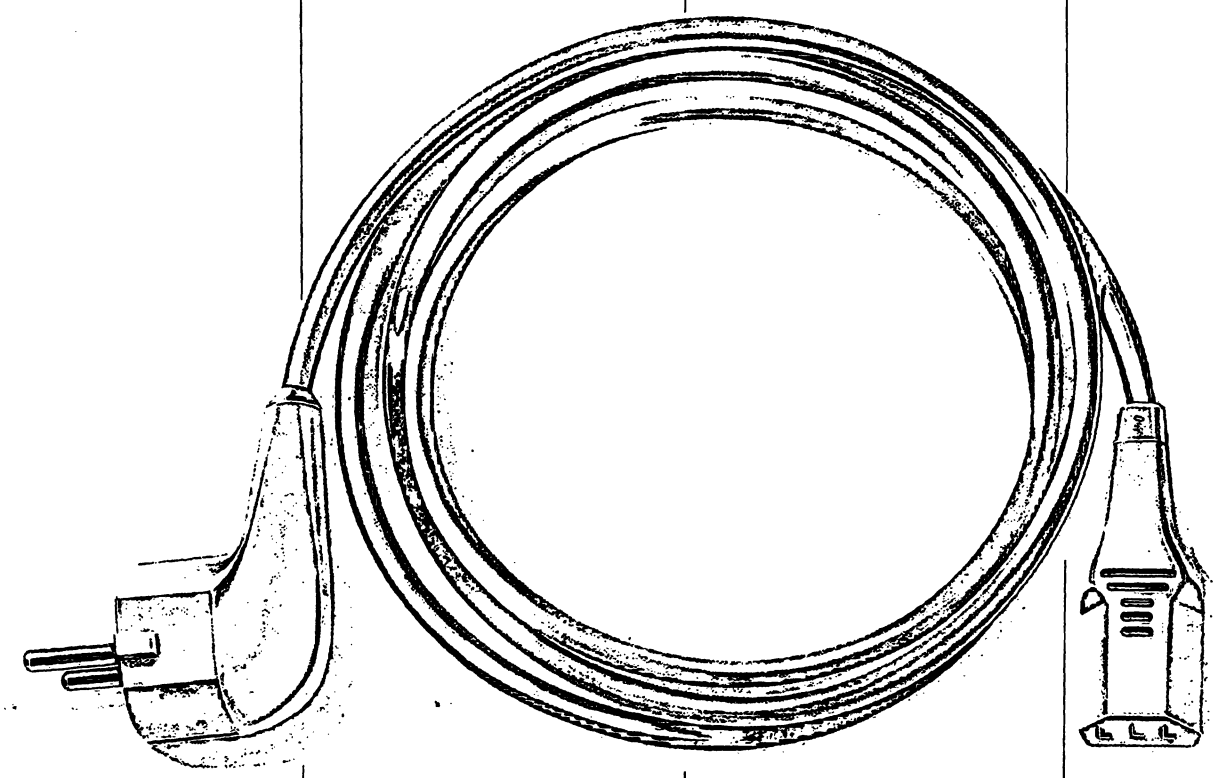

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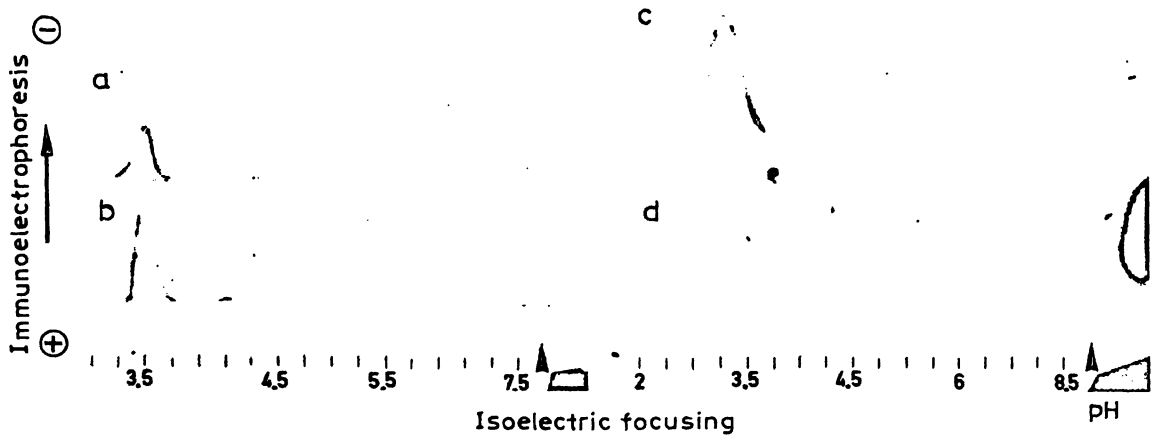

Fig. 2. Effects of Danazol therapy on C1-inhibitor in EDTA-plasma from patients with hereditary angioedema: Type I = a, b; Type IIa = c, d.
a = pretreatment sample, C1-inhibitor antigen level = 0.04 g/l
b = one month of therapy, C1-inhibitor antigen level = 0.09 g/l
c = pretreatment sample, C1-inhibitor antigen level = 0.24 g/l
d = one month therapy, C1-inhibitor antigen level = 0.46 g/l

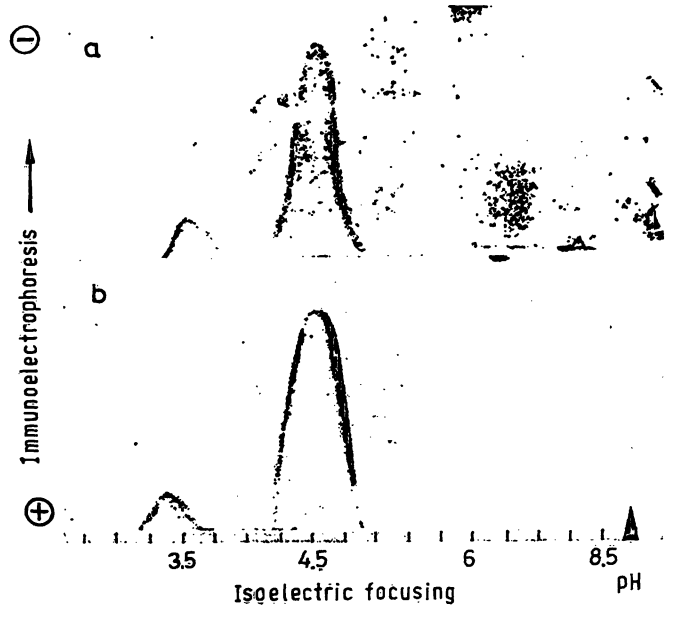

Fig. 3. Effects of Danazol therapy on C1-inhibitor in EDTA-plasma from a patient with Type IIb hereditary angioedema with a high C1-inhibitor antigen level.
a = pretreatment samples, C1-inhibitor antigen level = 0.62 g/l (sample dilution 1 : 2)
b = one month treatment, C1-inhibitor antigen level = 1.2 g/l (sample dilution 1 : 4)

Discussion

From this study we conclude that C1-inhibitor is a very acidic protein, with an isoelectric point of 3.5–3.6, which is intermediate between those previously reported by *Haupt* (16) and *Curd* (12). The charge on the protein did not change during storage at −80 °C for 8 months. All our patients with hereditary angioedema had normal C1-inhibitor at pH 3.5–3.6. In type IIa, with normal antigenic levels of C1-inhibitor, we could not differentiate functionless protein from normal C1-inhibitor.

In 3 patients (type IIb) with C1-inhibitor antigen levels 2–4 times the normal value, the majority of antigenic material was focused at pH 4.5–4.6 and immunoprecipitated both with anti-C1-inhibitor and anti-albumin, suggesting the presence in the plasma of a functionless complex C1-inhibitor-albumin as previously reported (17). In all 3 patients, the C1-inhibitor in pretreatment plasma was indistinguishable from the C1-inhibitor obtained during Danazol treatment, which increased the synthesis of both the dysfunctional C1-inhibitor and normal C1-inhibitor gene products.

The fact that in type IIa disease both normal and functionless protein have the same charge confirms that the chemical difference responsible for the dysfunction must be very small (probably a few amino acid substitutions), since it does not cause any detectable change in size or antigenicity.

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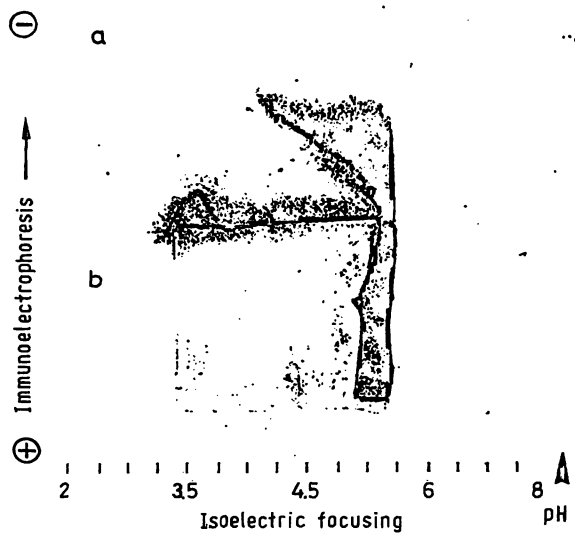

Fig. 4. Disappearance of the major peak focused at pH 4.5—4.6 in plasma from a patient with type IIb hereditary angioedema in an anti-albumin intermediate gel.
a: gel containing anti-albumin antibody
b: gel containing anti-C1-inhibitor antibody

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